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The Mediterranean recluse spider *Loxosceles rufescens* (Dufour, 1820) (Araneae: Sicariidae) established in a natural cave in Thailand

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Abstract. *Loxosceles rufescens* (Dufour, 1820), the Mediterranean recluse spider, is a cosmopolitan species with toxic venom which can occasionally cause dermatological injuries in humans. Here, we report the finding of *L. rufescens* through intensive survey and exploration of six natural limestone caves in the western region of Thailand. These data provide the first direct evidence of *L. rufescens* living in large numbers in a natural habitat outside of their native Mediterranean range. Although the currently known distribution of *L. rufescens* in Thailand is quite narrow (the spiders were only found in one of the six caves explored), data on their biology and local habitat preferences are provided to better understand the colonization requirements of this species in the target area.

Keywords: Cave, distribution, toxic, violin spider

The genus *Loxosceles* Heineken & Lowe, 1832, the recluse or violin spiders, is one of two genera in the family Sicariidae (Araneae), comprising 107 species distributed around the world (Platnick 2015). They are notorious for their bites, which occasionally produce a suite of symptoms known as 'loxoscelism', characterised by severe necrotie dermatologic injuries (Swanson & Vetter 2006). One of the most well-known recluse species is the Mediterranean recluse spider, *L. rufescens* (Dufour, 1820). The original range of this species is in the circum-Mediterranean region, however it has been spread widely, most likely through accidental human transportation (Vetter 2008). It is now found in many temperate and tropical areas including the islands of the Atlantic, Madagascar, the Hawaiian islands, Australia, Mexico, and the United States (Gertsch & Ennik 1983). In Asia, *L. rufescens* has been reported in India (Tikader 1963), China (Chen & Gao 1990; Chen & Zhang 1991; Song et al. 1999), Russia (Dunin 1992), Taiwan (Song et al. 1999), Japan (Yaginuma 1940, 1986; Yoshikura 1987; Ono 2009), and South Korea (Namkung 2002). A recent molecular phylogenetic study of the *L. rufescens* complex suggested that *L. rufescens* might have occurred in Malaysia, although limited numbers of specimens were used for the study (only one female and two males) (Duncan et al. 2010).

Here, we report the finding of a large population of *L. rufescens* in a natural habitat in Southeast Asia. We document the presence of *L. rufescens* in western Thailand, provide observations on its morphology, natural history, habitat and identification, and propose how it may have arrived in Thailand.

METHODS

We surveyed six natural limestone caves located in the Khao Wang Khmer area of Kanchanaburi province in the western part of Thailand (14°25′N, 98°52′E) on 18 April and 25 May 2015 (Figs. 1, 2). These caves are located in the conservation area of the Plant Genetic Conservation Project under the Royal Initiative of Prineess Maha Chakri Sirindhorn (RSPG). Spiders were hand collected and specimens were preserved in 95% ethanol and transferred to the Center of Excellence in Entomology, Chulalongkorn University (Bangkok) for dissection and identification.

Spiders that appeared to be *L. rufescens* were examined to eonfirm their identity, and to provide morphological data on the population found in Thailand. The genitalia of females were dissected and cleared with a 3M KOH aqueous solution. An Olympus SZ60 stereoscope coupled with an Olympus digital camera (Camedia c-4040 zoom) was used to photograph the diagnostic features of the putative *L. rufescens*. All measurements (in millimeters) were carried out under an ocular micrometer in a stereomicroscope (Olympus: Zeiss Stemi DV4). Descriptions are based on one specimen for each sex; however, for the leg measurements (left legs), we also displayed measurements for an additional 5♀ and 4♂ speeimens (Table 1).

The following abbreviations are used throughout the text: AME, anterior median eye/s; CL, carapace length; CW, earapace width (measured at the widest point); ED, endite; LA, labium; LE, lateral eye/s; SL, sternum length; SW, sternum width (measured at the widest point); and TL, total length.

Specimens were identified in accordance with Greene et al. (2009). All voucher specimens (i.e., 10 ♀, 4 ♂ and 6 subadult juveniles) are deposited at the Chulalongkorn University Museum of Zoology (CUMZ), Bangkok (Thailand) for future analysis.

SYSTEMATICS

Family Sicariidae Keyserling, 1880
Genus *Loxosceles* Heineken & Lowe, 1832

Loxosceles Heineken & Lowe, 1832 in Lowe, 1832: 321. Full synonymy: see Gertsch & Ennik (1983) and Platnick (2015).

Figure 1.—The 'Death Railway' trail (black dashed line), which is in close proximity to Tum-Wangpra cave (red spot) where specimens of *Loxosceles rufescens* were found. Yellow spots are caves that we explored without finding any *L. rufescens*. The inset picture on the top right is indicative of what remains of the railway nowadays, whereas the inset picture on the bottom right shows the locality of Tum-Wangpra cave.

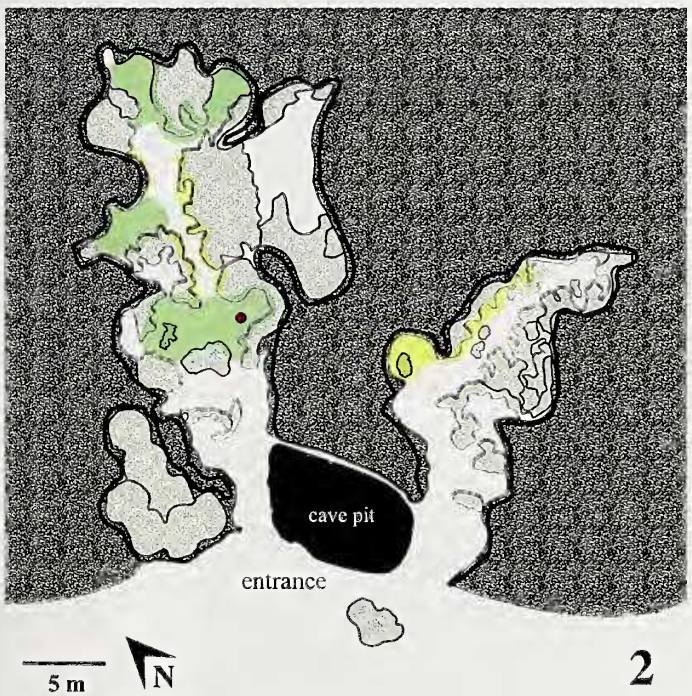


Figure 2.— A schematic outline of Tum-Wangpra cave located in the Khao Wang Khmer area of Kanchanaburi province, Thailand. Light green shading indicates areas where more than 100 *L. rufescens* individuals were found (residing mostly under scattered rocks on the ground), whereas yellow shading indicates areas with less than 100 individuals (and most specimens hiding in crevices on the cave walls). Dark grey areas depict the limestone cave boundaries, and light grey areas illustrate small limestone boulders that are accessible by humans. The red spot corresponds with the red arrow in Fig. 6, showing the exact location of the spider aggregation site.

Type Species.—*Scytodes rufescens* Dufour, 1820, by subsequent designation of Bonnet (1957).

Remarks.—As is typical of the genus *Loxosceles*, all specimens examined from Thailand possessed the following suite of characters: carapace flattened, longer than wide with a deep fovea (Fig. 5); clypeus porrect (Fig. 5); legs long and slender; sternum longer than wide; and abdomen oblong bearing spine-like setae (Lotz 2012). A diagnostic character for the females of *L. rufescens* is the structure of the spermathecae, which are characterized by the presence of closely-spaced receptacles and wide, laterally brown, sclerotized copulatory tubes (Fig. 3). These features were found in all 10 female specimens examined. In addition, when we examined four male specimens, the palpal tibia length to height ratio was less than 2.0, the cymbium was about half to less than half the length of the tibia, the length of the cymbium was similar to that of the pulpal bulb, and the palpal bulb was globular with a thin embolus (Fig. 4). These male characters are generally diagnostic of *L. rufescens* (Lotz 2012), although not exclusively so (Duncan et al. 2010; Planas & Ribera 2015; Planas et al. 2015).

Loxosceles rufescens (Dufour, 1820)
(Figs. 3–5, 8, 9)

Scytodes rufescens Dufour, 1820: 203, pl. 76, fig. 5. Full synonymy: see Platnick (2015).

Material examined.—THAILAND: *Kanchanaburi*: 6♀, 3 subadult juveniles, Sai Yok district, Tum-Wangpra cave, 14°24′47″N, 98°51′43″E, 18 April 2015, hand collected, N. Chomphuphuang (CUMZ-AR-ARA-Sic.2015.1–9); 4 ♂, 4♀,

Table 1.—Left leg and palp measurements of a single representative adult female and adult male of *Loxosceles rufescens* from Thailand, and averages and standard deviations of five additional females and four male specimens (all measurements are in millimeters). Leg formulas are provided.

	I	II	III	IV	Palp
Female (CUMZ-AR-ARA-Sic.2015.1); leg formula: 2413					
Femur	5.04	5.40	4.8	5.52	1.35
Patella	1.11	1.17	0.99	0.90	0.33
Tibia	5.28	6.56	4.80	5.46	0.87
Metatarsus	5.70	6.00	5.10	6.60	-
Tarsus	1.38	1.25	1.00	1.25	1.08
Total	18.51	20.38	16.69	19.73	3.63
Male (CUMZ-AR-ARA-Sic.2015.10); leg formula: 2413					
Femur	5.12	7.00	4.74	5.52	1.41
Patella	1.17	1.05	0.90	1.05	0.30
Tibia	6.80	8.40	5.90	5.92	0.90
Metatarsus	6.63	8.63	6.10	7.25	-
Tarsus	1.50	1.74	1.20	1.65	0.60
Total	21.22	26.82	18.84	21.39	3.21
Five adult females (CUMZ-AR-ARA-Sic.2015.2–6); leg formulas: 2143					
Femur	4.29±0.99	4.56±0.99	3.96±0.86	4.38±0.99	0.93±0.32
Patella	0.90±0.21	0.87±0.36	0.92±0.19	0.86±0.17	0.37±0.05
Tibia	3.72±1.85	4.23±2.21	3.14±1.51	3.59±1.75	0.53±0.22
Metatarsus	4.28±1.28	4.70±1.36	4.06±1.07	4.44±1.37	-
Tarsus	1.38±0.16	1.39±0.13	1.20±0.20	1.23±0.24	1.00±0.22
Total	15.24±3.54	16.52±3.96	13.78±3.07	15.13±3.29	2.65±0.42
Four adult males (CUMZ-AR-ARA-Sic.2015.10–13); leg formulas: 2143					
Femur	5.06±0.55	6.18±0.95	4.54±0.56	4.88±0.82	1.05±0.24
Patella	1.02±0.14	0.94±0.30	1.08±0.35	0.96±0.08	0.38±0.10
Tibia	6.15±0.86	7.40±1.16	4.85±0.87	5.28±0.76	0.55±0.26
Metatarsus	5.86±0.72	7.26±1.35	5.30±0.79	6.29±0.83	-
Tarsus	1.45±0.13	1.51±0.20	1.25±0.06	1.44±0.25	0.40±0.14
Total	14.51±8.78	17.11±10.84	12.91±7.97	14.05±8.60	1.95±1.21

3 subadult juveniles, same data except 25 May 2015 (CUMZ-AR-ARA-Sic.2015.10–20).

Description.—Carapace flattened, longer than wide, with a deep fovea and porrect clypeus (Fig. 5); chelicerae joined basally, with an immovable, thumb-like extension on the medial apical surface and short fangs; six eyes in three diads; legs long and slender, with two tarsal claws bearing serrated bristles on a small onychium; female genitalia haplogyne, with single broad opening and two spermathecae.

Female (CUMZ-AR-ARA-Sic.2015.1): TL = 8.1; CL = 3.6, CW = 3.2; eye diameter 0.15; AME-LE = 0.27; eye row strongly recurved; abdomen length = 4.26, width = 3.0; clypeus height = 0.32, SL = 1.88, SW = 1.59; LA = 0.78 long, 0.66 wide; ED = 1.29 long, 0.36 wide; leg formula: 2413; leg and palp measurements shown in Table 1. Carapace and chelicerae light brown, anterior carapace with dark brown 'violin' pattern (Fig. 5); legs and palps pale yellow to orange covered by short black setae, female palp without claw; coxae and sternum pale

Figures 3–4.—Diagnostic characters of *Loxosceles rufescens*: 3, female spermathecae (dorsal view); 4, male pedipalp. Scale bars = 0.15 mm (Fig. 3), 0.5 mm (Fig. 4).

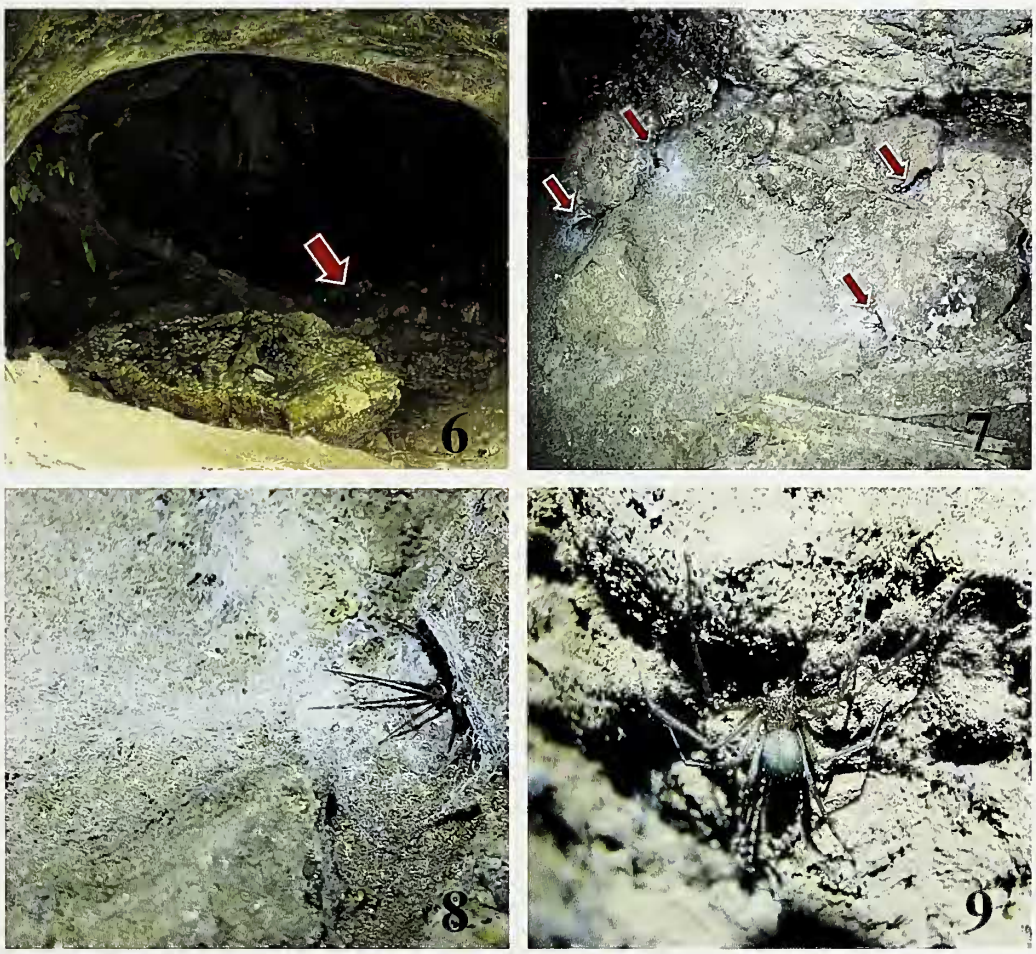
Figure 5.—Adult female *Loxosceles rufescens* carapace (dorsal view). Scale bar = 1 mm.

yellow, labium and endites brown; abdomen yellowish, covered with setae. Receptacles of spermathecae closely spaced; copulatory tubes wide with lateral brown sclerotized area (Fig. 3).

Female variation: A single female specimen (CUMZ-AR-ARA-Sic.2015.2) has two black spots on the posterior end of the carapace; subadults or recently molted individuals with pale pigment in the violin pattern.

Male (CUMZ-AR-ARA-Sic.2015.10): TL = 5.10; CL = 2.68, CW = 2.40; eye diameter 0.09; AME-LE = 0.21; eye row strongly recurved; abdomen length = 3.00, width = 1.55; clypeus = 0.27; SL = 1.23, SW = 1.35; LA = 0.45 long, 0.51 wide; ED = 0.90 long, 0.33 wide; leg formula: 2413 (see discussion); leg and palp measurements shown in Table 1. Overall body and coloration similar to female except for the abdomen, which is distinctly smaller than that of the female. Palpal tibia length to height ratio less than 2.0; cymbium half of tibia length; cymbium as long as length of palpal bulb; bulb globular and embolus thin (Fig. 4).

Eggs: In the laboratory, we retrieved a silken egg sac from one female nine days after the collection date. The sac contained 24 eggs with egg diameters between 1.07–1.15 mm. The eggs took 23 days after the egg sac was deposited to hatch



Figures 6–9.—Tum-Wangpra limestone cave, the locality where *Loxosceles rufescens* specimens were collected: 6, entrance to the cave (red arrow indicates the area where numerous *L. rufescens* were found); 7, cave surface (red arrows indicate *L. rufescens* retreats on the limestone ground); 8, flocculent web of *L. rufescens* on the crevice of the limestone ground; 9, *L. rufescens* clinging to the cave wall.

into spiderlings under an ambient temperature of 27°C and 70% relative humidity. Only 21 individuals successfully hatched (87% hatch rate). We measured the total body length of the spiderlings two days after they emerged from the egg sac, at which time they were between 1.20–1.68 mm (average 1.46 mm).

Habitat: Of the six limestone caves and peripheral urban areas that we surveyed, *L. rufescens* were found in only one of the caves locally known as "Tum-Wangpra" (Fig. 2). This cave has two major areas that extended approximately 20–25 m from the cave entranee. The accessible area for humans is about 340 m^2 with an average height of 5 m above ground. The cave is partially accessible by sunlight at a distance of 5 m from the cave entrance, which is devoid of the spiders. The surface of the ground inside the cave is slightly warm (annual average air temperature = 27°C and annual average relative humidity = 81.6 %), and mainly covered with a dry loamy sand and bat guano (Fig. 6). A population of the Old World hog-nosed bat, *Craseonycteris thonglongyai* Hill, 1974 is also found inhabiting the cave. An intensive survey revealed that the spiders were aggregated in similar microhabitats throughout the cave (Figs. 7–9); they hid themselves in crevices on the walls (Fig. 9), or under scattered rocks on the ground (Fig. 7–8) where they made small retreats of flocculent silk. Some individuals of *L. rufescens* also clung on rocks beneath other spiders' webs. By counting the visible spiders, we determined that there might be approximately 500 or more *L. rufescens* individuals living in the cave.

DISCUSSION

L. rufescens is a cosmopolitan species widely distributed throughout the world (Platnick 2015). Although native to the Mediterranean region, the species has been spread to other areas by human activities (Harvey 1996). In Israel, *L. rufescens* is moderately common in houses and basements (Shulov et al. 1962). Greene et al. (2009) suggested that populations of the genus *Loxosceles* in the United States tend to be extremely dense in favorable urban environments such as steam tunnels and subterranean habitats. In Iran, *L. rufescens* is also found inside buildings and under rocks and logs in urban areas (Zamani & Rafinejad 2014). In the natural environment, *Loxosceles* populations can be found in caves and/or cavern-like habitats (see Newlands 1975; Gertsch & Ennik 1983; Griffin 1998; Ferreira et al. 2005; Gonçalves-de-Andrade et al. 2007). Here, we report the anomalous discovery of *L. rufescens* in a natural habitat in Thailand. Until now, *L. rufescens* has never been reported in a natural habitat outside of its native range in the Mediterranean region. Clearly, the description of the physical parameters of the cave in which the spiders were found, and an understanding of their basic biology, are important considerations for determining the colonization requirements of this species in the target area.

Although speculative, the occurrence of *L. rufescens* in this part of Thailand might be explained by passive transportation by humans during World War II. The area in which we discovered *L. rufescens* was called the 'Hellfire Pass', when it was a major route for the construction of the infamous "Death Railway" or the Burma-Siam Railway along the Mae Klong River in Kanchanaburi province (Waterford 1994). The entrance of Tum-Wangpra cave is in close proximity to the railway (Fig. 1); material for the construction of the railway may have harbored the spiders, since specific railing material had to be shipped from Japan during that period, and there were already reports of *L. rufescens* present in Japan before 1940 (Bösenberg & Strand 1906; Strand 1918; Yaginuma 1940).

In North and South America, studies on the biology, distribution, and medical aspects of *Loxosceles* are ongoing, particularly for the brown recluse spider *L. reclusa* Gertsch & Mulaik, 1940, which is endemic to the USA (Swanson and Vetter 2005). In contrast, research on the genetic diversity and venom potency of *L. rufescens* has only been extensively studied in recent years (Planas & Ribera 2015; Planas et al. 2015). A protein expression analysis of the sphingomyelinase D (SMase D) protein, which is considered to be the major component of *Loxosceles* venom that causes dermatological injuries (Binford et al., 2008), suggested that the SMase D protein activity in *L. rufescens* venom is as high as in other *Loxosceles* species (Planas et al. 2015). In 2014, it was reported that a man in Phrae province in the northern part of Thailand had been bitten by *L. reclusa* and died (Bangkokpost 2014). The symptoms reported were very similar to loxoscelism, and this news sparked much attention and in some cases hysteria from the general public, much of it unwarranted. However, a doctor later concluded that the man died as a result of a secondary bacterial infection due to an unidentified spider's bite, and not loxoscelism as originally thought. This hypothesis agrees with our survey of the village perimeter near where the man was bitten, which did not yield any *L. reclusa*. Thus, in Thailand there is still no verified report of a human being bitten by a *Loxosceles* spider.

Our next goal is to survey the distribution of *L. rufescens* beyond the Khao Wang Khmer area to identify the true range of the species. Molecular studies of the Thai *L. rufescens* are equally important for determining the spider's origins. Indeed, since genetic diversity can be high within speeies of *Loxosceles* that share similar spermathecal and palp morphologies (Duncan et al. 2010; Planas & Ribera 2015; Planas et al. 2015), our samples need to be tested to determine whether they are genetically consistent with *L. rufescens* s. s.

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